B36 37. (Amended Once) A host cell transfected with a first expression vector comprising a nucleic acid encoding a polypeptide of SEQ ID NO: 2 and a second expression vector comprising a nucleic acid encoding a polypeptide of SEQ ID NO: 12.

39. (Amended Once) A method of producing a soluble polypeptide complex of SEQ ID NO: 2 and SEQ ID NO: 12 comprising:

a) culturing the host cell of Claim 34 under conditions suitable for expression of the soluble polypeptide complex; and

b) isolating or purifying the soluble polypeptide complex.

B37 10

40. (Amended Once) A method of producing a soluble polypeptide complex of SEQ ID NO: 2 and SEQ ID NO: 12 comprising:

a) culturing the host cell of Claim 37 under conditions suitable for expression of the soluble polypeptide complex; and

b) isolating or purifying the soluble polypeptide complex.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached paG is captioned, "Version with markings to show changes made."

REMARKS

Claims 31-40 are pending. Claims 31, 37, 39, and 40, are amended. The Specification has been amended on the pages requested by the Examiner to add sequence identifiers. Applicants believe that no new matter is added by the foregoing amendments. Entry of these amendments is respectfully requested.

Since Applicants have fully and completely responded to the Examiner's Communication, this application is now in order for early action.

Applicants believe that no fees are due with this communication. Should this not be the case, the Commissioner is hereby authorized to debit any charges to DNAX Deposit Account No. 04-1239.

If the Examiner does not agree with any aspect of this Response, Applicants respectfully urge the Examiner to contact the undersigned by telephone.

Respectfully submitted,

Date: December 27, 2002

By: _____
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph on page 4, lines 2-18, has been amended as follows:

The present invention is directed to mammalian, e.g., primate or rodent, interleukin-B60 (IL-B60) (SEQ ID NO: 1 and 2 (human); SEQ ID NO: 3 and 4 (murine)) and its biological activities. It includes nucleic acids coding for polypeptides themselves and methods for their production and use. The nucleic acids of the invention are characterized, in part, by their homology to complementary DNA (cDNA) sequences disclosed herein, and/or by functional assays for growth factor- or cytokine-like activities, e.g., G-CSF (see Nagata (1994) in Thomson The Cytokine Handbook 2d ed., Academic Press, San Diego) and/or IL-6 (see Hirano (1994) in Thomson The Cytokine Handbook 2d ed., Academic Press, San Diego). Also provided are polypeptides, antibodies, and methods of using them, including using nucleic acid expression methods. Methods for modulating or intervening in the control of a growth factor dependent physiology or an immune response are provided.

The paragraph on page 5, lines 12-17, has been amended as follows:

The invention further provides a method of producing an antigen:antibody complex, comprising contacting, under appropriate conditions, a primate IL-B60 polypeptide (SEQ ID NO: 2) with an antibody that specifically or selectively binds the polypeptide of the invention, thereby allowing the complex to form.

The paragraph on page 6, lines 3-10, has been amended as follows:

Methods are provided, e.g., of producing an antigen:antibody complex, comprising contacting, under appropriate conditions, a primate complex comprising IL-B60 (SEQ ID NO: 2) CLF-1 polypeptides (SEQ ID NO: 12) with an antibody that selectively or specifically binds to an isolated soluble complex

comprising the mature protein portion of SEQ ID NO: 2 or 4, and the mature protein portion of SEQ ID NO: 12 or 13, thereby allowing the complex to form.

The paragraph beginning on page 6, line 15, and continuing to page 7, line 28, has been amended as follows:

The invention also provides a composition of matter selected from: an isolated polypeptide comprising at least seven amino acids identical to segments of SEQ ID NO: 2 or 4; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 2 or 4; a natural sequence polypeptide comprising mature SEQ ID NO: 2 or 4; or a fusion polypeptide comprising IL-B60 (SEQ ID NO: 2 or 4) sequence. In certain embodiments, the distinct nonoverlapping segments of identity include: one of at least eight amino acids; one of at least five amino acids and a second of at least six amino acids; at least three segments of at least four, five, and six amino acids, or one of at least twelve amino acids. In other embodiments the polypeptide of the composition of matter: is the polypeptide which: comprises a mature sequence of Table 1; is an unglycosylated form of IL-B60 (SEQ ID NO: 2 or 4); is from a primate, such as a human; comprises at least seventeen amino acids of SEQ ID NO: 2 or 4; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 2 or 4; is a natural allelic variant of IL-B60 (SEQ ID NO: 2 or 4); has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate IL-B60 (SEQ ID NO: 2); is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; is a deletion or insertion variant from a natural sequence; or which further comprises: at least seven amino acids identical to segments of SEQ ID NO: 12 or 13; at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 12 or 13; a natural sequence polypeptide comprising mature SEQ ID NO: 12 or 13; or a primate CLF-1 (SEQ ID NO: 12).

In additional preferred embodiments, the composition comprises: a substantially pure IL-B60 (SEQ ID NO:1, 2, 3, 4) and CLF-1 (SEQ ID NO: 12 or 13); a sterile IL-B60 polypeptide (SEQ ID NO: 2 or 4) comprising the mature protein of SEQ ID NO: 2 or 4; or the described polypeptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration. The invention provides fusion polypeptides which comprise: mature protein sequence of Table 1; a detection or purification tag, including a FLAG, His6, or Ig sequence; or sequence of another cytokine receptor family protein, including CLF-1 (SEQ ID NO:12). Kit embodiments include those comprising the polypeptide of the composition and: a compartment comprising the protein or polypeptide; or instructions for use or disposal of reagents in the kit.

The paragraph on page 8, lines 6-23, has been amended as follows:

Related binding compounds include those comprising an antigen binding site from an antibody that specifically or selectively binds to a natural polypeptide, as described above, wherein: the binding compound is in a container; the IL-B60 polypeptide is from a human; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a mature polypeptide of Table 1; is raised against a mature IL-B60 (SEQ ID NO: 2 or 4); is raised to a purified human IL-B60 (SEQ ID NO:2); is immunoselected; is a polyclonal antibody; binds to a denatured IL-B60 (SEQ ID NO: 2 or 4); exhibits a Kd to antigen of at least 30 μM; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Kits are provided comprising such a binding compound and: a compartment comprising the binding compound; or instructions for use or disposal of the reagents of the kit.

The paragraph on page 8, lines 24-34, has been amended as follows:

Methods are provided for producing an antigen:antibody complex, comprising contacting, under appropriate conditions, a primate IL-B60 polypeptide (SEQ ID NO:2) with a described antibody, thereby allowing the complex to form. Preferably, in the method: the complex is purified from other cytokines; the complex is purified from other antibody; the contacting is with a sample comprising a cytokine; the contacting allows quantitative detection of the antigen; the contacting is with a sample comprising the antibody; or the contacting allows quantitative detection of the antibody.

The paragraph on page 9, lines 6-25, has been amended as follows:

Nucleic acid embodiments include an isolated or recombinant nucleic acid encoding the described polypeptide, wherein: the IL-B60 is from a human (SEQ ID NO: 1, 2); or the nucleic acid: encodes an antigenic peptide sequence of Table 1; encodes a plurality of antigenic peptide sequences of Table 1; encodes a plurality of antigenic peptide sequences of Table 4; exhibits identity over at least thirteen nucleotides to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a primate; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the IL-B60 (SEQ ID NO: 2 or 4); or is a PCR primer, PCR product, or mutagenesis primer. Preferred embodiments include where the isolated or recombinant nucleic acid is in a cell or tissue. The cell may be: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell.

The paragraph on page 9, lines 26-30, has been amended as follows:

Kits are provided comprising the described nucleic acid and: a compartment comprising the nucleic acid; a compartment further comprising a primate IL-B60 polypeptide (SEQ ID NO: 2); or instructions for use or disposal of reagents in the kit.

The paragraph on page 10, lines 3-11, has been amended as follows:

In a further aspect, the invention provides a nucleic acid which: hybridizes under wash conditions of 30 minutes at 30° C and less than 2M salt to the coding portion of SEQ ID NO: 1; or exhibits identity over a stretch of at least about 30 nucleotides to a primate IL-B60 (SEQ ID NO: 1). In preferred embodiments, the wash conditions that are at 45° C and/or 500 mM salt; or at 55° C and/or 150 mM salt; or the stretch is at least 55 nucleotides, e.g., at least 75 nucleotides.

The paragraph on page 10, lines 12-24, has been amended as follows:

Methods are provided, e.g., of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a mammalian IL-B60 (SEQ ID NO: 1, 2, 3, 4); or contacting the cell with an agonist or antagonist of a complex comprising mammalian IL-B60 (SEQ ID NO: 2 or 4) and CLF-1 (SEQ ID NO: 12 or 13). Additionally, the invention provides a method of increasing the secretion of: an IL-B60 (SEQ ID NO: 2 or 4) sequence, comprising expressing the polypeptide with CLF-1 (SEQ ID NO: 12 or 13); or a CLF-1 (SEQ ID NO: 12 or 13), comprising expressing the CLF-1 (SEQ ID NO: 12 or 13) with an IL-B60 (SEQ ID NO: 2 or 4) sequence. In preferred embodiments of the method, the increasing is at least 3 fold, 5X, 7X, 10X, or more; or the expressing is of a recombinant nucleic acid encoding one or both of the polypeptide and CLF-1 (SEQ ID NO: 12 or 13).

The paragraph beginning on page 10, line 34, and continuing to page 11, line 23, has been amended as follows:

Other embodiments of the invention include, e.g., an isolated soluble complex comprising at least 6 amino acids of the mature protein portion of SEQ ID NO: 2 or 4, and: at least 6 amino acids of the mature protein portion of SEQ ID NO: 12 or 13; or at least 6 amino acids of the mature protein portion of the

CNTF-R (SEQ ID NO: 9 or 10). Such complex may, e.g., comprise a recombinant polypeptide of mature SEQ ID NO: 2 or 4; comprise a recombinant polypeptide of mature SEQ ID NO: 12 or 13; comprise a recombinant polypeptide of mature CNTF-R (SEQ ID NO: 9 or 10); comprise both a recombinant polypeptide of mature SEQ ID NO: 2 or 4, and a recombinant polypeptide of mature SEQ ID NO: 12 or 13; comprise both a recombinant polypeptide of mature SEQ ID NO: 2 or 4, and a recombinant polypeptide of mature CNTF-R (SEQ ID NO: 9 or 10); be detectably labeled; be in a buffered solution; or be in a sterile solution. Preferred embodiments include those which: comprise a mature IL-B60 polypeptide (SEQ ID NO: 1 or 2); comprise a mature CLF-1 polypeptide (SEQ ID NO: 12 or 13); comprises a mature CNTF-R polypeptide (SEQ ID NO: 9 or 10); exhibit at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 2 or 4; exhibit epitopes from both primate L-B60 (SEQ ID NO: 2) and primate CLF-1 (SEQ ID NO: 12); exhibit epitopes from both primate L-B60 (SEQ ID NO: 2) and primate CNTF-R (SEQ ID NO: 9); not be glycosylated; be attached to a solid substrate; be conjugated to another chemical moiety; or comprise a detection or purification tag, including a FLAG, His6, or Ig sequence.

The paragraph beginning on page 11, line 27, and continuing to page 12, line 35, has been amended as follows:

Fusion polypeptides are provided, which include, e.g., an isolated or recombinant polypeptide comprising: a first segment comprising at least seven amino acids identical to segments of SEQ ID NO: 2 or 4, and a second segment comprising at least seven amino acids identical to segments of mature SEQ ID NO: 12 or 13; at least two distinct nonoverlapping segments of at least five amino acids identical to segments of mature SEQ ID NO: 2 or 4, and a third segment comprising at least seven amino acids identical to segments of mature SEQ ID NO: 12 or 13; at least one segment comprising at least seven amino acids identical to segments of mature SEQ ID NO: 2 or 4, and two distinct nonoverlapping segments of at least five amino acids identical to segments of

mature SEQ ID NO: 12 or 13; a first segment comprising at least seven amino acids identical to segments of SEQ ID NO: 2 or 4, and a second segment comprising at least seven amino acids identical to segments of mature primate CNTF-R (SEQ ID NO: 9); at least two distinct nonoverlapping segments of at least five amino acids identical to segments of mature SEQ ID NO: 2 or 4, and a third segment comprising at least seven amino acids identical to segments of mature primate CNTF-R (SEQ ID NO: 9); or at least one segment comprising at least seven amino acids identical to segments of mature SEQ ID NO: 2 or 4, and two distinct nonoverlapping segments of at least five amino acids identical to segments of mature primate CNTF-R (SEQ ID NO: 9). Certain embodiments include those wherein the distinct nonoverlapping segments of identity: include one of at least eight amino acids; include one of at least five amino acids and a second of at least six amino acids; include at least three segments of at least four, five, and six amino acids, or include one of at least twelve amino acids. Other embodiments include those which: comprise a mature IL-B60 sequence; comprise a mature CLF-1 sequence; comprise a mature CNTF-R (SEQ ID NO: 9 and 10) sequence; exhibit at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 2 or 4; have a length at least about 30 amino acids; exhibit epitopes from both primate IL-B60 (SEQ ID NO: 2) and primate CLF-1 (SEQ ID NO: 12); exhibits epitopes from both primate IL-B60 (SEQ ID NO: 2) and primate CNTF-R (SEQ ID NO: 9); are not glycosylated; have a molecular weight of at least 30 kD; be a synthetic polypeptide; be attached to a solid substrate; be conjugated to another chemical moiety; or comprise a detection or purification tag, including a FLAG, His6, or Ig sequence.

The paragraph on page 13, lines 1-11, has been amended as follows:

Other embodiments include a composition comprising: substantially pure combination of IL-B60 (SEQ ID NO: 2 or 4) and CLF-1 (SEQ ID NO: 12 or 13); substantially pure combination of IL-B60 (SEQ ID NO: 2 or 4) and CNTF-R (SEQ ID NO: 9 or 10); a sterile polypeptide described above; or the polypeptide described above and a carrier, wherein the carrier is: an aqueous compound,

including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration. A kit is provided comprising a polypeptide as described, and: a compartment comprising the polypeptide; and/or instructions for use or disposal of reagents in the kit.

The paragraph beginning on page 13, lines 20, and continuing to page 14, line 4, has been amended as follows:

Binding compounds are provided, e.g., comprising an antigen binding site from an antibody, which antibody specifically binds a described complex, but not to any of the mature polypeptides of SEQ ID NO: 2, 4, 12, 13, or CNTF-R (SEQ ID NO: 9 or 10). Certain embodiments include those wherein: the binding compound is: in a container; an Fv, Fab, or Fab2 fragment; or conjugated to another chemical moiety; or the antibody: is raised against a substantially pure complex of IL-B60 (SEQ ID NO: 2 or 4) with CLF-1 (SEQ ID NO: 12 or 13); is raised against a substantially pure complex of IL-B60 (SEQ ID NO: 2 or 4) with CNTF-R (SEQ ID NO: 9 or 10); is immunoselected; is a polyclonal antibody; exhibits a Kd to antigen of at least 30 μM; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Additional embodiments include a composition comprising: a sterile binding compound as described, or the binding compound as described and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

The paragraph on page 14, lines 5-20, has been amended as follows:

With the binding composition are provided a kit comprising the binding compound and: a compartment comprising the binding compound; or instructions for use or disposal of reagents in the kit. Also provided are methods of producing an antigen:antibody complex, comprising contacting under appropriate conditions a primate complex comprising: IL-B60 (SEQ ID NO: 2)

and CLF-1 polypeptides (SEQ ID NO: 12); or IL-B60 (SEQ ID NO: 2) and CNTF-R polypeptides (SEQ ID NO: 9 or 10); with an antibody as described, thereby allowing the complex to form. Preferably, in the method, the complex is purified from other cytokines; the complex is purified from other antibody; the contacting is with a sample comprising a cytokine; the contacting allows quantitative detection of the antigen; the contacting is with a sample comprising the antibody; or the contacting allows quantitative detection of the antibody.

The paragraph beginning on page 14, line 21, and continuing to page 15, line 21, has been amended as follows:

Various nucleic acids are provided, e.g., an isolated or recombinant nucleic acid: encoding the amino acid portions described above; encoding the amino acid portions as described, and comprise a segment at least 30 contiguous nucleotides from SEQ ID NO: 1 or 3; which will coexpress a segment of at least seven contiguous amino acids from SEQ ID NO: 2 or 4, and a segment of at least seven contiguous amino acids from SEQ ID NO: 12 or 13; or which will coexpress a segment of at least seven contiguous amino acids from SEQ ID NO: 2 or 4, and a segment of at least seven contiguous amino acids from CNTF-R (SEQ ID NO: 9 or 10). Preferred nucleic acids include those which, e.g.,: encode IL-B60 from a human (SEQ ID NO: 2); encode CLF-1 from a human (SEQ ID NO: 12); encodes CNTF-R (SEQ ID NO: 9) from a human; are an expression vector; further comprise an origin of replication; comprise a detectable label; comprise synthetic nucleotide sequence; or are less than 6 kb, preferably less than 3 kb. A cell comprising the recombinant nucleic acid is provided, e.g., wherein the cell is: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell. Various nucleic acid kits are provided, e.g., comprising the nucleic acid and: a compartment comprising the nucleic acid; a compartment further comprising a primate IL-B60 polypeptide (SEQ ID NO: 2); a compartment further comprising a primate CLF-1 polypeptide (SEQ ID NO: 12); a compartment further comprising a primate CNTF-R (SEQ ID NO: 9) polypeptide; or instructions

for use or disposal of reagents in the kit. Methods are also provided, e.g., of making a duplex nucleic acid, comprising contacting such a nucleic acid with a complementary nucleic acid under appropriate conditions, thereby forming said duplex; of expressing a polypeptide, comprising expressing the nucleic acid, thereby producing the polypeptide; or of transfecting a cell, comprising contacting said cell under appropriate conditions with the nucleic acid, thereby transfecting the cell.

The paragraph on page 15, lines 22-35, has been amended as follows:

In an alternative embodiment, the invention provides an isolated or recombinant nucleic acid which encodes at least 5 contiguous amino acids of SEQ ID NO: 12, 13, or primate CNTF-R (SEQ ID NO: 9) and: hybridizes under wash conditions of 30 minutes at 30° C and less than 2M salt to the coding portion of SEQ ID NO: 1; or exhibits identity over a stretch of at least about 30 nucleotides to a primate IL-B60 (SEQ ID NO: 1). Preferred embodiments include: the isolated nucleic acid, wherein: the contiguous amino acids number at least 8; the wash conditions are at 45° C and/or 500 mM salt; or the stretch is at least 55 nucleotides; or the recombinant nucleic acid, wherein: the contiguous amino acids number at least 12; the wash conditions are at 55° C and/or 150 mM salt; or the stretch is at least 75 nucleotides.

The paragraph on page 16, lines 1-15, has been amended as follows:

The invention particularly provides methods of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a complex comprising mammalian IL-B60 (SEQ ID NO: 2 or 4) and: CLF-1 (SEQ ID NO: 12 or 13); or CNTF-R (SEQ ID NO: 9 or 10). It also provides methods of producing the proteins, e.g., producing a complex described, comprising coexpressing a recombinant IL-B60 (SEQ ID NO: 2 or 4) with a recombinant CLF-1 (SEQ ID NO: 12 or 13) or CNTF-R (SEQ ID NO: 9 or 10); increasing the secretion of an IL-B60 polypeptide (SEQ ID NO: 2 or

4) comprising expressing the polypeptide with CLF-1 (SEQ ID NO: 12 or 13) or CNTF-R (SEQ ID NO: 9 or 10); or increasing the secretion of a CLF-1 (SEQ ID NO: 12 or 13) polypeptide, comprising expressing the CLF-1 (SEQ ID NO: 12 or 13) with an IL-B60 (SEQ ID NO: 2 or 4). Typically, the increasing is at least 3 fold; or the expressing is of a recombinant nucleic acid encoding one or both of the polypeptide and CLF-1 (SEQ ID NO: 12 or 13).

The paragraph on page 18, lines 2-17, has been amended as follows:

The present invention provides amino acid sequences and DNA sequences encoding various mammalian proteins which are cytokines, e.g., which are secreted molecules which can mediate a signal between immune or other cells. See, e.g., Paul (1998) Fundamental Immunology (4th ed.) Raven Press, N.Y. The full length cytokines, and fragments, or antagonists will be useful, e.g., in physiological modulation of cells expressing a receptor. It is likely that IL-B60 (SEQ ID NO: 2 or 4) has either stimulatory or inhibitory effects on hematopoietic cells, including, e.g., lymphoid cells, such as T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc. The proteins will also be useful as antigens, e.g., immunogens, for raising antibodies to various epitopes on the protein, both linear and conformational epitopes.

The paragraph on page 18, lines 18-21, has been amended as follows:

A sequence encoding IL-B60 (SEQ ID NO: 2) was identified from a human genomic sequence. The molecule was designated huIL-B60. A rodent sequence, e.g., from mouse (SEQ ID NO: 4), is also described.

The paragraph on page 18, lines 23-30, has been amended as follows:

The human gene encodes a small soluble cytokine-like protein, of about 198 amino acids. The psort predicted signal sequence probably is about 17

residues, and would run from the Met to about Ala. See Table 1 and SEQ. ID. NO: 1 and 2. IL-B60 (SEQ ID NO: 2 or 4) exhibits structural motifs characteristic of a member of the long chain cytokines. Compare, e.g., IL-B60 (SEQ ID NO: 1, 2, 3, 4), G-CSF, and IL-6, sequences available from GenBank. Closest matching is with CT-1, oncostatin M, and CNTF. See also Table 2.

The paragraph on page 23, lines 1-2, has been amended as follows:

Table 2: Comparison of primate (SEQ ID NO: 1) and rodent (SEQ ID NO: 4) embodiments of IL-B60, both the nucleotide and amino acid sequences.

The paragraph on page 24, lines 3-6, has been amended as follows:

Alignment of IL-B60 (SEQ ID NO: 2 (human); SEQ ID NO: 4 (mouse): underlined are proposed helices. In general, those residues that are in helix A and D and not pointing inward toward the core (mostly the hydrophobic residues in A and D helix) are the most likely residues to interact with receptors.

The paragraph on page 35, lines 10-31, has been amended as follows:

An IL-B60 polypeptide (SEQ ID NO: 2 or 4) that specifically binds to or that is specifically immunoreactive with an antibody, e.g., such as a polyclonal antibody, generated against a defined immunogen, e.g., such as an immunogen consisting of an amino acid sequence of SEQ ID NO: 2 or fragments thereof or a polypeptide generated from the nucleic acid of SEQ ID NO: 1 is typically determined in an immunoassay. Included within the metes and bounds of the present invention are those nucleic acid sequences described herein, including functional variants, that encode polypeptides that selectively bind to polyclonal antibodies generated against the prototypical IL-B60 polypeptide (SEQ ID NO: 2 or 4) as structurally and functionally defined herein. The immunoassay typically uses a polyclonal antiserum which was raised, e.g., to a protein of SEQ ID NO: 2. This antiserum is selected, or depleted, to have low crossreactivity against

appropriate other closely related family members, preferably from the same species, and any such crossreactivity is removed by immunoabsorption or depletion prior to use in the immunoassay. Appropriate selective serum preparations can be isolated, and characterized.

The paragraph beginning on page 35, line 32, and continuing to page 36, line 25, has been amended as follows:

In order to produce antisera for use in an immunoassay, the protein, e.g., of SEQ ID NO: 2, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An appropriate host, e.g., an inbred strain of mice such as Balb/c, is immunized with the protein of SEQ ID NO: 2 using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane). Alternatively, a substantially full length synthetic peptide derived from the sequences disclosed herein can be used as an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, e.g., a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against other closely related family members, e.g., LIF, CT-1, CNTF, DIL-40, or other members of the IL-6 family, using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably at least two IL-6/IL-12 family members are used in this determination in conjunction with the target. These long chain cytokine family members can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein. Thus, antibody preparations can be identified or produced having desired selectivity or specificity for subsets of IL-B60 family members. Alternatively, antibodies may be prepared which bind to the complex comprising the IL-B60 (SEQ ID NO: 2 or 4) with the CLF-1 (SEQ ID NO: 12 or 13).

The paragraph beginning on page 37, line 22, and continuing to page 38, line 13, has been amended as follows:

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI. Sequence identity changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The conservation may apply to biological features, functional features, or structural features. Homologous amino acid sequences are typically intended to include natural polymorphic or allelic and interspecies variations of a protein sequence. Typical homologous proteins or peptides will have from 25-100% identity (if gaps can be introduced), to 50-100% identity (if conservative substitutions are included) with the amino acid sequence of the IL-B60 (SEQ ID NO: 2 or 4). Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.

The paragraph beginning on page 38, line 14, and continuing to page 39, line 7, has been amended as follows:

The isolated IL-B60 DNA (SEQ ID NO: 1 or 3) can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of short nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, antigenic, or other functional activity. These

modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. "Mutant IL-B60" encompasses a polypeptide otherwise falling within the sequence identity definition of the IL-B60 (SEQ ID NO: 2 or 4) as set forth above, but having an amino acid sequence which differs from that of IL-B60 (SEQ ID NO: 2 or 4) as normally found in nature, whether by way of deletion, substitution, or insertion. This generally includes proteins having significant identity with a protein having sequence of SEQ ID NO: 2, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the natural full length disclosed sequences. Full length sequences will typically be preferred, though truncated versions will also be useful, likewise, genes or proteins found from natural sources are typically most desired. Similar concepts apply to different IL-B60 proteins (SEQ ID NO: 2 or 4), particularly those found in various warm blooded animals, e.g., mammals and birds. These descriptions are generally meant to encompass all IL-B60 proteins (SEQ ID NO: 2 or 4), not limited to the particular primate embodiments specifically discussed.

The paragraph on page 39, lines 8-35, has been amended as follows:

IL-B60 (SEQ ID NO: 1, 2, 3, 4) mutagenesis can also be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See, e.g., Sambrook, et al. (1989); Ausubel, et al. (1987 and Supplements); and Kunkel, et al. (1987) Methods in Enzymol. 154:367-382. Preferred embodiments include, e.g., 1-fold, 2-fold, 3-fold, 5-fold, 7-fold, etc.,

preferably conservative substitutions at the nucleotide or amino acid levels. Preferably the substitutions will be away from the conserved cysteines, and often will be in the regions away from the helical structural domains. Such variants may be useful to produce specific antibodies, and often will share many or all biological properties. See Table 2. Recognition of the cytokine structure provides important insight into the structure and positions of residues which may be modified to effect desired changes in receptor interaction. Also, the interaction of the IL-B60 (SEQ ID NO: 2 or 4) with the CLF-1 (SEQ ID NO: 12 or 13) protein requires complementary structural features in the interacting surface.

The paragraph on page 43, lines 4-22, has been amended as follows:

This invention also contemplates the use of derivatives of IL-B60 proteins (SEQ ID NO: 2 or 4) other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties or protein carriers. Covalent or aggregative derivatives will be useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of binding partners, e.g., other antigens. An IL-B60 (SEQ ID NO: 2 or 4) can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-IL-B60 antibodies or an alternative binding composition. The IL-B60 proteins (SEQ ID NO: 2 or 4) can also be labeled with a detectable group, e.g., for use in diagnostic assays. Purification of IL-B60 (SEQ ID NO: 2 or 4) may be effected by an immobilized antibody or complementary binding partner, e.g., binding portion of a receptor.

The paragraph beginning on page 43, line 23, and continuing to page 44, line 3, has been amended as follows:

A solubilized IL-B60 (SEQ ID NO: 2 or 4) or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for binding. Purified antigen can be used to screen monoclonal antibodies or antigen-binding fragments, encompassing antigen binding fragments of natural antibodies, e.g., Fab, Fab', $F(ab)_2$, etc. Purified IL-B60 (SEQ ID NO: 2 or 4) antigens can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the cytokine, which may be diagnostic of an abnormal or specific physiological or disease condition. This invention contemplates antibodies raised against amino acid sequences encoded by nucleotide sequence shown in SEQ ID NO: 1, or fragments of proteins containing it. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific domains, e.g., helices A, B, C, or D of the IL-B60, or the Ig domains of the CLF-1 (SEQ ID NO: 12 or 13).

The paragraph beginning on page 48, line 34, and continuing to page 49, line 8, has been amended as follows:

The described peptide sequences and the related reagents are useful in detecting, isolating, or identifying a DNA clone encoding IL-B60 (SEQ ID NO: 2 or 4), e.g., from a natural source. Typically, it will be useful in isolating a gene from a mammal, and similar procedures will be applied to isolate genes from other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of IL-B60 (SEQ ID NO: 1 or 3) from the same, e.g., polymorphic variants, or other species. A number of different approaches will be available to successfully isolate a suitable nucleic acid clone.

The paragraph on page 49, lines 17-23, has been amended as follows:

For example, a specific binding composition could be used for screening of an expression library made from a cell line which expresses an IL-B60 (SEQ ID NO: 1, 2, 3, 4). Screening of intracellular expression can be performed by

various staining or immunofluorescence procedures. Binding compositions could be used to affinity purify or sort out cells expressing a surface fusion protein.

The paragraph on page 83, lines 2-23, has been amended as follows:

To identify the signaling receptors for IL-B60/CLF-1 conditioned medium from hIL-B60 (SEQ ID NO: 1 or 2) and mCLF-1 (SEQ ID NO: 13) cotransfected 293T cells was added to BA/F3 cells stably transfected with human gp130 alone or hgp130 in combination with the hIL-6R, hOSMR, hLIFR, or hLIFR and hCNTFR, respectively. Only BA/F3 cells expressing gp130, LIFR, and CNTFR showed a proliferative response upon stimulation with IL-B60/CLF-1. To analyze the possibility of a signaling complex consisting of CNTFR/gp130 or CNTFR/LIFR only, two soluble fusion proteins were designed connecting either the CNTFR or CLF-1 to IL-B60 via a flexible linker. Similar so-called hyper-cytokines have been shown to be 100-1000x more active on cells than cytokine and soluble receptor added separately. See Fischer, et al. (1997) Nature Biotechnol. 15:142-145. Hyper-CNTFR-IL-B60 was able to induce proliferation of BAF3/gp130/LIFR cells but not of BAF3/gp130 cells, showing that the LIFR is a component of the signaling complex. Stimulation of cells with hyper-CLF-1-IL-B60 did not result in proliferation of any cell line. This indicated that although necessary for IL-B60 (SEQ ID NO: 2 or 4) secretion, CLF-1 (SEQ ID NO: 12 or 13) is not a subunit of the active signaling receptor complex.

In the claims:

31. (Amended Once) An isolated or recombinant nucleic acid encoding a soluble cytokine polypeptide complex comprising the amino acid sequence of:

a) IL-B60 having a sequence of SEQ ID NO: 2 [or 4]; and

b) CLF-1 having a sequence of SEQ ID NO: 12 [or 13].

32. (Reiterated) The nucleic acid of Claim 31, wherein the nucleic acid is detectably labeled.

33. (Reiterated) An expression vector comprising the nucleic acid of Claim 31.

34. (Reiterated) A host cell comprising the expression vector of Claim 33.

35. (Reiterated) The host cell of Claim 34, wherein the host cell is:

a) a prokaryotic cell;

b) a mammalian cell;

c) an insect cell; or

d) a yeast cell.

36. (Reiterated) The nucleic acid of Claim 31, wherein the nucleic acid of ILB60 comprises SEQ ID NO: 1 or 3.

37. (Amended Once) A host cell transfected with a first expression vector comprising a nucleic acid encoding a polypeptide of SEQ ID NO: 2 [or 4] and a second expression vector comprising a nucleic acid encoding a polypeptide of SEQ ID NO: 12 [or 13].

38. (Reiterated) The host cell of Claim 37, wherein the host cell is:

a) a prokaryotic cell;

b) a mammalian cell;

c) an insect cell; or

d) a yeast cell.

39. (Amended Once) A method of producing a soluble polypeptide complex of SEQ ID NO:2 [or 4] and SEQ ID NO:12 [or 13] comprising:

a) culturing the host cell of Claim 34 under conditions suitable for expression of the soluble polypeptide complex; and

b) isolating or purifying the soluble polypeptide complex.

40. (Amended Once) A method of producing a soluble polypeptide complex of SEQ ID NO:2 [or 4] and SEQ ID NO:12 [or 13] comprising:

a) culturing the host cell of Claim 37 under conditions suitable for expression of the soluble polypeptide complex; and

b) isolating or purifying the soluble polypeptide complex.